Eur J Plant Pathol (2009) 124:331–335
DOI 10.1007/s10658-008-9410-2

Evaluation of glyphosate-tolerant soybean cultivars for resistance to bacterial pustule

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Received: 24 August 2008 / Accepted: 27 November 2008 / Published online: 10 December 2008

Abstract *Xanthomonas axonopodis* pv. *glycines* causes bacterial pustule of soybean, which is a common disease in many soybean-growing areas of the world and is controlled by a single recessive gene (*rxp* gene) commonly found in many conventional glyphosate-sensitive soybean cultivars. Since glyphosate-tolerant cultivars are commonly planted today, there has been no information about whether these new cultivars have bacterial pustule resistance. The goal of this study was to screen glyphosate-tolerant soybean cultivars for resistance to *X. axonopodis* pv. *glycines*. Three experiments were completed to evaluate resistance. In experiment 1, 525 commercial glyphosate-tolerant cultivars from 2001 were inoculated with *X. axonopodis* pv. *glycines* strain UIUC-1. Following inoculation, many of the cultivars were resistant (developed no detectable pustule symptoms) although 152 (~29%) developed bacterial pustule. In experiment 2, the aggressiveness of three strains (UIUC-1, UIUC-2, and ATCC 17915) of *X. axonopodis* pv. *glycines* were compared on three bacterial pustule-susceptible, glyphosate-tolerant cultivars. One strain (UIUC-1) was less aggressive than the other two (UIUC-2 and ATCC 17915) on all three cultivars examined. In experiment 3, 45 cultivars from 2005 (all different from 2001) were inoculated with *X. axonopodis* pv. *glycines* ATCC 17915. A range of disease severities developed with five cultivars (11%) having disease severity ratings as high as or higher than those on a susceptible check cultivar. Overall, these results suggested that resistance to bacterial pustule occurs in glyphosate-tolerant soybean cultivars, but not at 100% frequency, which means bacterial pustule outbreaks could occur when a susceptible cultivar is planted and conditions are conducive for bacterial pustule development.

Keywords *Xanthomonas axonopodis* pv. *glycines* · Roundup Ready® soybeans · Foliar bacterial pathogen

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Xanthomonas axonopodis pv. *glycines* (Vauterin et al. 1995, 2000) causes bacterial pustule of soybean (*Glycine max*). Bacterial pustule is a foliar disease and is common in soybean-producing countries such as Brazil, China, India, and the USA (Ansari 2005; Hartman and Wang 1997; Sinclair 1999; Wrather et al. 2001a). *Xanthomonas axonopodis* pv. *glycines* is spread by wind-blown rain and infects through stomata and wounds on leaves (Sinclair 1999). This bacterium also survives in contaminated crop residues, and seed-borne

transmission is common (Groth and Braun 1989). Under conditions of high rainfall, humidity, and temperature, disease development is promoted. Symptoms include yellow-to-brown lesions with pustules on the underside of leaves which may merge to form large necrotic areas leading to premature shedding of leaves (Fig. 1).

Bacterial pustule is a disease controlled by maintaining resistance (*rxp* gene) in cultivar development (Hartwig and Lehman 1951; Narvel et al. 2001). The *rxp* gene, a recessive gene originally derived from the cv. CNS, has been widely used to develop commercial soybean cultivars with resistance to bacterial pustule (Hartwig and Lehman 1951; Narvel et al. 2001; Sinclair 1999). Nonetheless, bacterial pustule continues to reduce soybean yields worldwide (Wrather et al. 2001a, b; Wrather and Koenning 2006). Most of the commercial soybean cultivars grown today are glyphosate-tolerant; in this regard, approximately 60% and 87% of the commercial soybean cultivars planted in the world and in the USA are glyphosate-tolerant, respectively (USDA 2007; Cerdeira and Duke 2006; Duke and Cerdeira 2007). The development of resistance to bacterial pustule in these commercial soybean cultivars is unknown. Therefore, the objective was to screen glyphosate-tolerant soybean cultivars for resistance to three strains of *X. axonopodis* pv. *glycines*.

Glyphosate-tolerant soybean cultivars were selected at random from available seed lots in the 2001 and 2005 Varietal Information Programme for Soybeans (VIPS) at the University of Illinois at Urbana-Champaign (UIUC). Control cultivars consisted of Spencer or PI 520733 (both susceptible to bacterial pustule) and Williams 82 (resistant to bacterial pustule; Bernard and Lindahl 1972); all of these cultivars are sensitive to glyphosate. Plants were grown in the greenhouse in plastic trays (16 or 18 plants per tray; 2 or 3 plants per cultivar per tray) containing SB300 Universal Professional Growing Mix (Sun Gro, Bellevue, WA), fertilizer (Oscomote 19-6-12), and a covering of coarse vermiculite. Greenhouse conditions were: temperature (25°C); light source (1,000-watt fixtures); photoperiod (14-h light, fixtures on; 10-h dark, fixtures off); humidity (100%); and water (twice daily). All plants were grown for 2 weeks prior to inoculation.

Fig. 1 Symptoms of bacterial pustule, caused by *Xanthomonas axonopodis* pv. *glycines*, on soybean leaves

Strains of *X. axonopodis* pv. *glycines* were obtained from the National Soybean Pathogen Collection Centre at UIUC and included UIUC-1 isolated in 2001 from bacterial pustule on the leaves of field-grown PI 520733 at UIUC and UIUC-2 isolated in 2001 from bacterial pustule on the leaves of field-grown Spencer at UIUC. Both organisms were isolated by streaking directly from pustules that had been macerated in a small amount of sterile water onto potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) plates and incubated at 25°C for 48 h. Isolates were picked and repeatedly streaked on PDA to establish pure cultures; UIUC-1 and UIUC-2 were Gram-negative rods which produced yellow, mucoid colonies on PDA and were capable of producing bacterial pustule on greenhouse-grown PI 520733. *Xanthomonas axonopodis* pv. *glycines* ATCC 17915 (originally isolated from a soybean leaf) was obtained from the American-Type Culture Collection (ATCC; Manassas, VA). The identity of all three of these organisms was confirmed to the species level as *X. axonopodis* by an independent laboratory (Bacterial Identification & Fatty Acid Analysis Laboratory, Plant Pathology Department, University of Florida, Gainesville, FL) using cellular fatty acid-based analysis.

For experiment 1, *X. axonopodis* pv. *glycines* UIUC-1 was grown in 250-ml culture flasks containing 100 ml of potato dextrose broth (Difco) for 8 h at 25°C on a shaker at 240 rpm. Following incubation, cells were harvested by centrifugation, washed in sterile deionised water, repelleted by centrifugation, and resuspended in sterile deionized water to a final $OD_{600\ nm}$ of 0.3 (~10^8 viable cells ml^{-1}). Prior to

inoculation, leaflets of the trifoliolate leaves were each damaged five times with a 5-prong perforation needle to mimic natural wounding. Plants (one or two per cultivar) were inoculated by spraying 20 ml of the cell suspension on 16 plants using an atomiser until runoff. After inoculation (7 days), plants were examined for lesions characteristic of bacterial pustule (Fig. 1), and the number of pustules on each leaflet was counted and recorded.

For all subsequent plant inoculations (i.e., experiments 2 and 3), cell suspensions were prepared by growing strains (UIUC-1, UIUC-2, and ATCC 17915) of *X. axonopodis* pv. *glycines* in 125-ml culture flasks containing 50 ml of sterile broth culture medium for 14–16 h at 30°C on a shaker at 175 rpm. This semi-defined medium (pH 7) contained (mg l^{-1}): glucose, 4,500; yeast extract, 1,000; K_2HPO_4, 500; $(NH_4)_2SO_4$, 1,000; KH_2PO_4, 500; NaCl, 450; $MgSO_4{\cdot}7H_2O$, 250; $CaCl_2{\cdot}2H_2O$, 50; tri-Na nitrilotriacetate, 15; $MnSO_4{\cdot}H_2O$, 5; $FeSO_4{\cdot}7H_2O$, 1; $CO(NO_3)_2{\cdot}6H_2O$, 1; $ZnCl_2$, 1; $NiCl_2{\cdot}6H_2O$, 0.1; H_2SeO_3, 0.01; $CuSO_4{\cdot}5H_2O$, 0.1; $AlK(SO_4)_2{\cdot}12H_2O$, 0.1; H_3BO_3, 0.1; $Na_2MoO_4{\cdot}2H_2O$, 0.1; and $Na_2WO_4{\cdot}2H_2O$, 0.1. Following incubation, cell suspensions (20 ml) of each strain were prepared as described above and supplemented with 320 mesh carborundum (0.1 g) and Triton X-100 (5 µl). Plants (three per cultivar per strain of *X. axonopodis* pv. *glycines*), were inoculated by spraying 20 ml of a cell suspension on 18 plants using an atomiser until runoff. Negative controls (three per cultivar) were plants sprayed with water containing carborundum and Triton X-100 but lacking *X. axonopodis* pv. *glycines*. After inoculation (14 days), plants were examined, and the number of pustules or overall disease severity (based on number, size, and intensity of pustules) was recorded.

In experiment 1, 152 (~29%) of 525 glyphosate-tolerant soybean cultivars screened for resistance to bacterial pustule were susceptible to UIUC-1 and developed bacterial pustule. Based on the average number of pustules per plant, glyphosate-tolerant cultivars were divided into three groups. Highly susceptible cultivars (64) averaged between 161 and 44 pustules per plant, with an overall mean ± standard error of 65±2 pustules per plant, and were placed in this group since these cultivars formed more pustules per plant than the susceptible control PI 520733 which averaged 43 pustules per plant. Moderately susceptible cultivars (45) averaged between 41 and 20 pustules per plant, with an overall mean ± standard error of 31±1 pustules per plant, and were placed in this group since these cultivars had fewer pustules per plant than the highly susceptible control PI 520733. The resistant control Williams 82 averaged 19 pustules per plant, and was classified as moderately resistant. Moderately resistant cultivars (43) also formed pustules but unlike Williams 82 averaged between 18 and 2 pustules per plant, with an overall mean of ten pustules per plant ± 1 standard error. The remainder of the cultivars (373) displayed no bacterial pustules and were classified as resistant. Our tests were greenhouse evaluations, and the resistance in Williams 82 was at least partially overcome presumably due to the high inoculum load. This has been reported before on the original source of resistance (cv. CNS) which developed chlorotic lesions when inoculated (Chamberlain 1962).

In experiment 2, glyphosate-tolerant soybean cvs Wilkens 2582 RR, DKB 26-51, and DKB 32-52, all susceptible to bacterial pustule in experiment 1, were inoculated with UIUC-1, UIUC-2, and ATCC 17915. As observed in experiment 1, all three of these soybean cultivars displayed some level of pustule development at the 1st and 2nd trifoliolate stages 2 weeks after inoculation (Fig. 2). Furthermore, bacterial pustule developed on plants regardless of the strain of *X. axonopodis* pv. *glycines* used for inoculation indicating that the susceptibility of glyph-

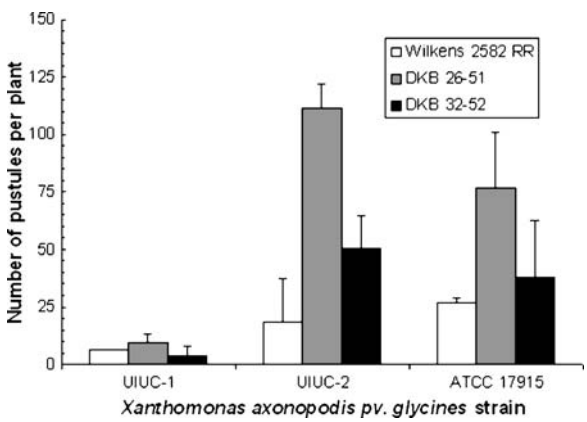

Fig. 2 Evaluation of three glyphosate-tolerant soybean cultivars from 2001 (Wilkens 2582 RR, DKB 26-51, and DKB 32-52) for resistance to three strains (UIUC-1, UIUC-2, and ATCC 17915) of *Xanthomonas axonopodis* pv. *glycines*. Values represent the means ± standard errors

Table 1 Evaluation of 45 glyphosate-tolerant soybean cultivars for resistance to *Xanthomonas axonopodis* pv. *glycines*, the cause of bacterial pustule[1]

Bacterial pustule severity ranking[2]	Number[3]	Cultivar
5 (most severe)	5 (11%)	Stine 3532-4, Agsource 9362, NK S37-N4, FS HISOY HS 3936, FS HISOY HS 3916
4	11 (24%)	Dekalb DKB 26-52, Dekalb 31-51, Kruger 349, Dekalb DKB 36-52, Lewis 3875, Trisler 3833, Garst 3712, Asgrow AG 3906, Pioneer 93M93, Pioneer 93M90, Midwest Seed Gen Gr 3331
3	12 (27%)	Pioneer 93M10, Pioneer 93M11, Fontanelle 8153, Asgrow AG 2705, Pioneer 92M70, Stine 2702-4, Garst 2903, Pioneer 92M92, FS HISOY HS 3536, Garst 3512, Dekalb DKB 38-52, Agventure 39J3
2	11 (24%)	Asgrow AG 2801, Garst 2812, Pioneer 92M80, Becks 323, Pioneer 93M30, Willcross 2331N, Pioneer 93M50, Asgrow AG 3602, FS HISOY HS 3726, Garst 3624, Asgrow AG 3905
1 (least severe)	6 (13%)	Asgrow AG 3101, Pioneer 92M91, Garst 3212, Stine 3242-4, Asgrow AG 3305,LG Seeds C 3444

[1] Plants (three per cultivar) were inoculated with *X. axonopodis* pv. *glycines* ATCC 17915.

[2] Bacterial pustule severity (based on number, size, and intensity of pustules; see Fig. 3 for severity rankings) among soybean cultivars was ranked from 5 to 1, with 5 being the most severe and 1 being the least severe. Bacterial pustule severity rankings for Spencer (susceptible control) and Williams 82 (resistant control) were 5 and 3, respectively.

[3] Number and percentage of cultivars at a given severity ranking.

osate-tolerant cultivars to bacterial pustule was not specific or unique to UIUC-1. Overall, pustule development was consistently lower with Wilkens 2582 RR than with DKB 26-51 or DKB 32-52 (Fig. 2). In addition, among the three strains of *X. axonopodis* pv. *glycines* tested, UIUC-2 and ATCC 17915 produced slightly more pustules on Wilkens 2582 RR, DKB 26-51, and DKB 32-52 plants than UIUC-1.

In experiment 3, 45 glyphosate-tolerant soybean cultivars from 2005 (all different from 2001) were inoculated with *X. axonopodis* pv. *glycines* ATCC 17915 (Table 1). This strain was selected for use in this experiment since it produced more pustules than UIUC-1 in the previous experiment (Fig. 2) and is commercially available and thus easily accessible to others involved with resistance screening of cultivars. All of the 2005 cultivars tested with ATCC 17915 developed bacterial pustule (Table 1), and, as before, a range in pustule severity was observed (Fig. 3). Indeed, 5 (11%) cultivars displayed very high pustule severity (i.e., symptoms equal to or greater than those observed with Spencer, a susceptible cultivar) while 17 (37%) cultivars were ranked as having pustule severity less than that observed on Williams 82 (resistant cultivar). In experiments 2 and 3, control plants (sprayed with sterile water lacking *X. axonopodis* pv. *glycines* but containing carborundum and Trition X-100) for each cultivar did not develop pustules during the duration of the experiments.

In summary, different strains of *X. axonopodis* pv. *glycines* UIUC-1, UIUC-2, and ATCC 17915 were capable of pustule formation on a wide variety of glyphosate-tolerant soybean cultivars. Differences

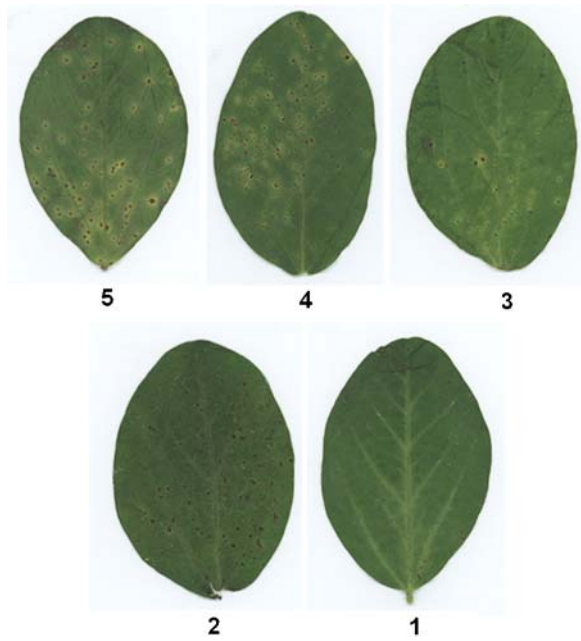

Fig. 3 Bacterial pustule severity rankings. Severity of disease symptoms (based on number, size, and intensity of pustules) was ranked from 5 to 1 with 5 being the most severe and 1 being the least severe. Bacterial pustule severity rankings for Spencer (susceptible control) and Williams 82 (resistant control) were 5 and 3, respectively

were observed between (i) strains of *X. axonopodis* pv. *glycines* relative to their potential to form bacterial pustule and (ii) glyphosate-tolerant soybean cultivars relative to their level of resistance to bacterial pustule. Reasons for the apparent susceptibility of many current commercial cultivars of glyphosate-tolerant soybeans to bacterial pustule are presently unknown. One possibility mentioned earlier is that resistance to bacterial pustule is not being maintained (selected for) as a trait during the development of some commercial glyphosate-tolerant soybean cultivars. In the process of developing transgenic soybean cultivars, the *rxp* gene (Hartwig and Lehman 1951), as well as other resistance genes (Manjaya and Pawar 1999; Sharma et al. 1993), might be lost or a susceptible allele introduced (Narvel et al. 2001). This in turn would increase the level of susceptibility to bacterial pustule in soybean cultivars. Another possibility is that the three strains of *X. axonopodis* pv. *glycines* used in this study consisted of extreme pathogenic variants. In this regard, variations in pathogenicity have been noted among isolates of *X. axonopodis* pv. *glycines* with some being highly virulent to a number of soybean cultivars (Ansari 2005; Kaewnum et al. 2005).

Acknowledgements A portion of this work was done during a sabbatical leave (S. L. Daniel) at the National Soybean Research Centre at the University of Illinois at Urbana-Champaign. We express our appreciation to Andrea Pabon, Curt Hill, Ron Warsaw, and Theresa Herman for their help on this project. Funding for this project was provided by the Illinois Soybean Association and the Tiffany Graduate Fund at Eastern Illinois University.

References

Ansari, M. M. (2005). Variability in Indian isolates of *Xanthomonas campestris* pv. *glycines*, the incitant of bacterial pustule of soybean (*Glycine max*). *Indian Journal of Agricultural Sciences*, *75*, 117–119.

Bernard, R. L., & Lindahl, D. A. (1972). Registration of Williams soybean. *Crop Science*, *12*, 716.

Cerdeira, A. L., & Duke, S. O. (2006). The current status and environmental impacts of glyphospate-resistant crops: a review. *Journal of Environmental Quality*, *35*, 1633–1658. doi:10.2134/jeq2005.0378.

Chamberlain, D. W. (1962). Reaction of resistant and susceptible soybeans to *Xanthomonas phaseoli* var. *sojensis*. *Plant Disease Reporter*, *46*, 707–709.

Duke, S. O., & Cerdeira, A. L. (2007). Risks and benefits of glyphosate-resistant crops. *ISB News Reports, January*, 1–5. Retrieved August 24, 2008 from http://nbiap.biochem.vt.edu/.

Groth, D. E., & Braun, E. J. (1989). Survival, seed transmission, and epiphytic development of *Xanthomonas campestris* pv. *glycines* in the north-central United States. *Plant Disease*, *73*, 326–330. doi:10.1094/PD-73-0326.

Hartman, G. L., & Wang, T. C. (1997). Bacterial pustule development and associated yield loss on nine soybean lines. *Proceedings from the World Soybean Research Conference V*, 21–27. February, 1994, Chiang Mai, Thailand (pp. 194–196).

Hartwig, E. E., & Lehman, S. G. (1951). Inheritance of resistance to the bacterial pustule disease in soybeans. *Agronomy Journal*, *43*, 226–229.

Kaewnum, S., Prathuangwong, S., & Burr, T. J. (2005). Aggressiveness of *Xanthomonas axonopodis* pv. *glycines* isolates to soybean and hypersensitivity responses by other plants. *Plant Pathology*, *54*, 409–415. doi:10.1111/j.1365-3059.2005.01176.x.

Manjaya, J. G., & Pawar, S. E. (1999). New genes for resistance to *Xanthomonas campestris* pv. *glycines* in soybean [*Glycine max* (L.) Merr.] and their inheritance. *Euphytica*, *106*, 205–208. doi:10.1023/A:1003560402719.

Narvel, J. M., Jakkula, L. R., Phillips, D. V., Wang, T., Lee, S.-H., & Boerma, H. R. (2001). Molecular mapping of *Rxp* conditioning reaction to bacterial pustule in soybean. *The Journal of Heredity*, *92*, 267–270. doi:10.1093/jhered/92.3.267.

Sharma, A., Nair, P. M., & Pawar, S. E. (1993). Identification of soybean strains resistant to *Xanthomonas campestris* pv. *glycines*. *Euphytica*, *67*, 95–99. doi:10.1007/BF00022730.

Sinclair, J. B. (1999). Bacterial pustule. In G. L. Hartman, J. B. Sinclair, & J. C. Rupe (Eds.), *Compendium of Soybean Diseases* (pp. 6–7). St. Paul, Minnesota, USA: American Phytopathological Society.

USDA (2007). National Agricultural Statistics Service (NASS), Agricultural Statistics Board, U.S. Department of Agriculture. Retrieved August 24, 2008 from http://www.usda.gov/nass/PUBS/TODAYRPT/acrg0607.pdf.

Vauterin, L., Hoste, B., Kersters, K., & Swings, J. (1995). Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology*, *45*, 472–489.

Vauterin, L., Rademaker, J., & Swings, J. (2000). Synopsis on the taxonomy of the genus *Xanthomonas*. *Phytopathology*, *90*, 677–682. doi:10.1094/PHYTO.2000.90.7.677.

Wrather, J. A., & Koenning, S. R. (2006). Estimates of disease effects on soybean yields in the United States 2003–2005. *Journal of Nematology*, *38*, 173–180.

Wrather, J. A., Anderson, T. R., Arsyad, D. M., Tan, Y., Ploper, L. D., Porta-Puglia, A., et al. (2001a). Soybean disease loss estimates for the top ten soybean-producing countries in 1998. *Canadian Journal of Plant Pathology*, *23*, 115–121.

Wrather, J. A., Stienstra, W. C., & Koenning, S. R. (2001b). Soybean disease loss estimates for the United States from 1996 to 1998. *Canadian Journal of Plant Pathology*, *23*, 122–131.